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METHOD FOR EARLY JUDGMENT OF COHESIVENESS [SOGYOSEI HANTEIHO]

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FOREIGN TITLE [54A]: SOGYOSEI HANTEIHO

Specification

[Scope of Patent Claims]

[Claim 1]

A method for judging early cohesiveness of raw material wheat which uses a fermentation test using yeast, with the invention being characterized as carrying out enzymatic processing of raw material wheat and using the enzymatically processed substance obtained as part or all of the raw material for the fermentation test.

[Claim 2]

A method for judging early cohesiveness of raw material wheat using a fermentation test which uses yeast, with the invention being characterized as carrying out enzymatic processing of raw material wheat by adding an enzyme to the raw material wheat, separating the polymer fraction of the enzymatically processed substance obtained and using said polymer

fraction as part of the raw material for the fermentation test.

[Claim 3]

A method for judging early cohesiveness of raw material wheat by carrying out a fermentation test which uses yeast, with the invention being characterized as carrying out enzymatic processing of raw material wheat by adding an enzyme to the raw material wheat, adding the enzymatically processed substance obtained or the polymer fraction separated from said enzymatically processed substance to synthetic wheat juice and using it as a raw material for the fermentation test, measuring the turbidity of the fermentation test raw material after 48 hours, thereby judging the presence of the early cohesiveness factor in the raw material wheat.

[Claim 4]

A method for judging early cohesiveness of raw material wheat as described in any of Claims 1 through 3 wherein the enzymatic processing is carried out by

using an enzyme which is provided with at least a combination of α-amylase, β-amylase, β-glucanase and protease.

[Claim 5]

A method for early cohesiveness of raw material wheat as described in any of Claims 1 through 4 wherein the raw material wheat is barley.

[Detailed Description of Invention]

[0001]

[Field of Industrial Application]

The present invention is a method for early judgment of cohesiveness of raw material wheat used in the fermentation of beer, whisky and other alcoholic drinks, that is, it judges the presence or absence of an early cohesiveness factor in raw material wheat.

[0002]

[Prior Art]

Wheat malt which is represented by barley and wheat is used as a fermentation raw material. Beer (which includes "sparkling wine" which has a malt

ratio that is lower than that of beer under the Alcoholic Beverage Revenue Act), whisky and the like are alcoholic beverages made by fermentation using yeast.

[0003]

When alcoholic beverages having this type of malt as a raw material are produced, a phenomenon known as the "early cohesiveness phenomenon" is observed during the fermentation process. During the fermentation process, particularly, the latter phase of fermentation, regardless of whether the sugar content in the yeast can be used or not, this is known as the phenomenon wherein the yeast coheres and precipitates and the progress of the fermentation stops. When this phenomenon is seen, the fermentation is incomplete and it becomes an alcoholic beverage which does not meet standards. It is already well known that a great deal of damage is incurred when beer and other alcoholic beverages are brewed using early cohesive wheat as a raw material.

[0004]

After carrying out a great deal of research on solving the problem of the early cohesiveness phenomenon in the production of alcoholic beverages using this malt as a raw material, it has been very nearly ascertained that this early cohesiveness phenomenon originates in the raw material wheat and that the polymer acid polysaccharides contained in the malt are the cause. However, it was not known whether the factor known as the cause of the early cohesiveness phenomenon (hereinafter "early cohesiveness factor") was present in the raw material wheat or whether it was caused in the barley milling process. In addition, little research is currently being carried out on a method of solving the early cohesiveness phenomenon by eliminating the early cohesiveness factor.

[0005]

When barley is used as a raw material, the presence of the early cohesiveness factor in the

barley has conventionally been confirmed and only barley malt not causing the early cohesiveness phenomenon has been selected. The conventional method used to confirm the presence of the early cohesiveness factor in barley involved actually milling the barley on a small scale, preparing a wheat juice from the malt, actually fermenting the wheat juice using yeast and confirming the presence of the early cohesiveness factor in the barley on the basis of the progress of the fermentation.

[0006]

The above mentioned conventional method involved confirming the presence of the early cohesiveness factor in the barley by reducing the actual fermentation scale. Although it was satisfactory in terms of reliability, approximately 7 days after the wheat had been milled and the wheat juice had been prepared and approximately 8 days after the presence of the early cohesiveness factor had been confirmed in the barley on the basis of the progress of

fermentation making a total of approximately half a month was required to confirm the presence of the early cohesiveness factor in the barley.

[0007]

In addition, the dormancy of the barley and the water-sensitivity and the like had to be taken into consideration in milling the wheat. Since the barley could not be provided for milling immediately after it had been harvested, the above mentioned conventional method could not be applied to barley immediately after harvesting. When the barley could be milled two months after harvesting, it took approximately half a month for the presence of the early cohesiveness factor in the barley to be confirmed. As a result, period of approximately two months at the earliest was required to confirm the presence of early cohesiveness in the barley after harvesting. The presence of the early cohesiveness factor could not be judged early on for barley in particular immediately after harvest and

when barley with early cohesiveness was purchased the damage done to it was considerably great.

[0008]

[Problems Which the Present Invention is Intended to Solve]

It is an object of the present invention to provide a method of judging the early cohesiveness of raw material wheat, that is, the presence of the early cohesiveness factor in raw material wheat, which is much less complex and within a shorter period of time than the above mentioned conventional method.

[0009]

[Means Used to Solve the Problems]

In the process of carrying out research on the early cohesiveness phenomenon, the inventors found that the early cohesiveness factor bringing about the early cohesiveness phenomenon was not produced in the barley milling process but rather was originally present in the raw material wheat. This means that they found that when enzymatic processing was carried

out by adding a specific enzyme to the raw material wheat, the early cohesiveness factor was extracted without decomposing and if a fermentation test was carried out using a substance processed enzymatically and extracted, the early cohesiveness could be judged and they attained the present invention.

[0010]

This means that the present invention is a method for judging the early cohesiveness of raw material

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wheat in barley and the like using a fermentation test using yeast, the invention characterized as a method for judgment of early cohesiveness wherein an enzyme, preferably one that contains at least a combination of α-amylase, β-amylase, β-glucanase and protease is added to the raw material wheat and the raw material wheat is subjected to enzymatic processing, thereby providing part or all of the raw material which uses the enzymatically processed substance for the fermentation test.

[0011]

The present invention also relates to a method for the purpose of judging early cohesiveness of raw material wheat in barley and the like use of a fermentation test which uses yeast, the invention characterized as adding an enzyme to the raw material wheat and preferably an enzyme that contains at least a combination of α-amylase, β-amylase, β-glucanase and protease and subjecting the raw material wheat to enzymatic processing, separating the polymer fraction of the enzymatically processed substance obtained and making said polymer fraction part of the fermentation test raw material.

[0012]

The present invention is also a method used for the purpose of judging early cohesiveness of barley and other raw material wheat by carrying out a fermentation test using yeast, the invention characterized as adding an enzyme to raw material wheat and preferably an enzyme which is at least a

combination of α-amylase, β-amylase, β-glucanase and protease, subjecting the raw material wheat to enzymatic processing, adding the enzymatically processed substance obtained or the polymer fraction separated from said enzymatically processed substance to a synthetic wheat juice and using it as a raw material for the fermentation test, measuring the turbidity of the raw material for the fermentation test, thereby judging the presence of the early cohesiveness factor in the raw material wheat.

[0013]

[Mode of Working the Invention]

The present invention is not necessarily restricted to passage of a certain amount of time after harvesting before the barley is milled using barley, wheat and other types of raw material wheat and these may be used immediately after harvesting. In addition, an amount of approximately 20 g can be used as the amount of raw material wheat required for judging the early cohesiveness. However, approximately

50 g should be prepared to enhance the precision of the experiments.

[0014]

Enzymatic processing of the raw material wheat should be carried out efficiently and specifically at least 90 wt % should be pulverized until it passes through a sieve having hole diameter dimensions of 0.547 mm. Next, this raw material wheat pulverized product is suspended in water and preferably in warm water at approximately 40 to 60° C, then the enzyme (preparation) is added and enzymatic processing is carried out.

[0015]

Any enzyme may be added in the present invention as long as it has an action whereby the early cohesiveness factor is extracted from the raw material wheat without being decomposed. For example, an enzyme should be added which contains at least a combination of α-amylase, β-amylase, β-glucanase and protease.

[0016]

As long as the enzyme added has an enzymatic activity, a highly pure enzyme need not necessarily be used. It may be, for example, a mycobiont processed substance containing an enzyme and a mixed substance of an enzyme with a different action may also be used. However, conditions are required such that the raw material wheat pulverized substance is prevented from becoming sticky in water.

[0017]

Next, we shall provide a more detailed description of the enzyme used. The enzyme containing α-amylase and protease and β-glucanase should be Ceremix 6XMG (made by Novo Ltd.); the β-glucanase used should be derived from *Aspergillus niger* (commercial name: Finizym; made by Nov Ltd.); and the β-amylase used should be derived from barley (β-amylase; made by Sigma Ltd).

[0018]

The amount of enzyme added, the reaction temperature, the reaction time and other enzymatic

processing conditions change depending on the properties of the enzyme used. However, the reaction should be carried out under conditions where the enzymatic activity can be brought out to the fullest. When the above mentioned enzymes are used, it is sufficient to carry out the reaction at approximately 40 to 60° C for at least approximately 2 to 3 hours. However, needless to say, the temperature can be changed gradually in order to imitate the usual saccharification process.

[0019]

After filtration of the enzymatically processed substance obtained in this enzymatic processing has been carried out using filter paper and the like, the enzymatic activity is deactivated by boiling or other heating and is again filtered to remove the heat coagulates produced by heating. When the raw material wheat has early cohesiveness properties, the early cohesiveness factor is contained in the polymer fraction in this filtrate (hereinafter, "pseudo wheat

juice"). As a result, if the pseudo wheat juice is left as is or after mixing with a suitable amount of another fermentation raw material, the regular fermentation test is carried out using yeast so that the presence of the early cohesiveness factor in the raw material wheat can be judged.

[0020]

Further, after only the polymer fraction has been separated from the above mentioned pseudo wheat juice, part of the fermentation test raw material is added to another fermentation raw material and the fermentation test is carried out so that the presence of the early cohesiveness factor can be judged all the more accurately. A specific method used to separate this polymer fraction involves adding approximately twice the capacity of ethanol to the above mentioned pseudo wheat juice and stirring it for approximately 5 minutes so that the polymer fraction can be obtained as a precipitate. However, as long as the method of separating the polymer fraction from the above

mentioned pseudo wheat juice involves precipitation using the above mentioned ethanol, dialysis, ultrafiltration and the like and separating the polymer fraction, any method can be used. When the raw material wheat has early cohesiveness, the early cohesiveness is contained in this polymer fraction.

[0021]

Needless to say, the culture medium to which the polymer fraction is added for the fermentation test may be the usual wheat juice. However, a synthetic wheat juice (for example, Weinfurtner, F., et al., Brauwissenshaft, 14, 109 (1961) prepared using constituents other than malt (sugar, amino acid, inorganic acids and the like) should be used to reproduce the results exactly. An improved method of preparing the synthetic wheat juice of Weinfurtner and the like is indicated as follows.

[0022]

(Method of Preparing Synthetic Wheat Juice)

We prepared in advance solution A, solution B, solution C and solution D having the composition indicated below. Further, we used solution C and solution D to make stock solutions and stored them in a refrigerator so that they were measured out in a

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clean bench. Next, we mixed 700 ml of solution A; 50 ml of solution B; 5 ml of solution C; and 100 μl of solution D, adjusted these to 800 ml using distilled water and adjusted them to pH 5.7.

[0023]

Solution A:

D(-)-fructose	2 g
D(+)-glucose	8 g
sucrose	4 g
maltose hydrate	64 g
dextrin	27 g
cazamino acid	3.5 g
peptone	4 g
CaCl2 (anhydrous)	1 g
KCl	1 g

We dissolved these in distilled water, adjusted them to 700 ml and sterilized them in an autoclave.

[0024] Solution B

We dissolved 1 g of $MgSO_4 \cdot 7H_2O$ in distilled water, adjusted it to 50 ml and sterilized it in an autoclave.

[0025] Solution C

inositol	500 mg
(+)-calcium pantothenate	500 mg
nicotinic acid	50 mg
thiamine hydrochloride	50 mg
pyridoxine hydrochlorate	50 mg
(+) biotin	50 mg
uracil	25 mg
guanine	25 mg

We dissolved this in distilled water and adjusted it to 250 ml, then sterilized it using a filter.

[0026] Solution D

H_3BO_3	100 mg
$ZnSO_4 \cdot 7 H_2O$	100 mg
$MnCl_2 \cdot 4 H_2O$	100 mg
$FeCl_3$	50 mg
$CoSO_4 \cdot 5 H_2O$	10 mg
KI	10 mg

We dissolved this in distilled water, adjusted it to 1000 ml and sterilized it in an autoclave.

[0027]

The method used to judge after the fermentation test is completed can be the conventional method (for example, see K. Morimoto et al.; Rept. Res. Lab. Kirin Brewery Co.Ltd., 18, 63 (1975). The conventional method involves either using the above mentioned pseudo wheat juice as is as a raw material (culture medium) for the fermentation test or adding the above mentioned pseudo wheat juice or the above mentioned polymer fraction to the wheat juice obtained from the raw material wheat having no early cohesiveness, using it as a fermentation test raw material (culture medium), then fermenting it for 8 days at approximately 8° C using yeast, measuring the turbidity and the degree of saccharification in said raw material (culture medium) for the degree of growth of the yeast, thereby judging it comprehensively.

[0028]

Particularly when a polymer fraction is used for the fermentation test using yeast, the turbidity after fermenting for 48 hours at 20° C is measured so that an accurate judgment can be confirmed. In this case, the absorbance (OD800) at a wavelength of 800 μm is measured using a turbidity meter and compared with the value of DPF (Degree of Premature Flocculation) 48 found by subtracting test section OD800 from contrast section OD800. At this time, the turbidity should be compared by simultaneously testing normal wheat or early cohesive wheat as a comparative example.

[0029]

Next, we shall provide a detailed explanation of the present invention and use a practical example of it. However, it should by no means be construed that the present invention is restricted to this.

[0030]

[Practical Example of Invention]

We added 0.5 g each of a commercially available enzyme (Finizym; made by Novo Ltd.) containing β-

amylase and a commercially available enzyme (Ceremix 6XMG; made by Novo Ltd.) containing protease, α-amylase and β-glucanase and 500 units of β-amylase (β-amylase; made by Sigma Ltd.) to 300 ml of warm water which had been heated to 55° C. We added 50 g of barley which had been pulverized using a disk mill, stirred it so that it was uniform, maintained it for 3 hours at 55° C, completed the enzymatic processing and obtained an enzymatically processed substance.

[0031]

We stirred this enzymatically processed substance thoroughly, filtered it using filter paper (No. 2 made by Toyo Filter Paper Ltd.) and fractioned exactly 180 ml of the filtrate. We heated the 180 ml fractioned, boiled it until the amount of the solution had decreased to less than half, then adjusted the entire amount to 100 m and obtained a pseudo wheat juice by again filtering it in the same way. Following this, we added small amounts of 200 ml of ethanol incrementally while stirring this pseudo wheat juice, stirred it for

5 minutes, then centrifuged it. We discarded the supernatant, added 10 ml of boiling water to the precipitate, dissolved the precipitate and adjusted the entire amount to 25 ml. We centrifuged it again and submitted the supernatant (hereinafter, "barley polymer fraction extract") to the fermentation test.

[0032]

We added 20 ml of the above mentioned barley polymer fraction extract to 80 ml of the synthetic wheat juice prepared using the above mentioned method, adjusted it to pH 5.7 and used it as a test section. Then, we adjusted it to pH 5.7 using distilled water instead of the barley polymer fraction extract and used this as a contrast section. We added 0.35 g of beer yeast to these, then placed it in a fermentation tube (100 ml capacity) with a diameter of 27 ml and fermented it at 20° C. 48 hours later, we fractioned it in 2 ml increments from 5 cm from the surface of the solution and measured the OD800. We found the value DPF48 which indicates the degree of early

cohesiveness by subtracting the value of the test section from the value of the contrast section.

[0033]

Using as samples 7 types of barley whose early cohesiveness had been confirmed by milling the barley on site, we carried out the test as indicated above and studied the correlation with the actual early cohesiveness. Results are indicated in Table 1.

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As can be seen from the Table, there is a high correlation between the DPF48 value and the actual early cohesiveness and it was confirmed that when the DPF48 value was great, it was barley having the early cohesiveness factor. In addition, the reproducibility of these results was confirmed to be extremely high.

[0034]

[Table 1]

Sample No. for Raw Material Barley	**DPF48 in the Present Invention**	**Results of Milling Barley on Site**
1	0.29	No early cohesiveness

2	0.29	No early cohesiveness
3	0.36	No early cohesiveness
4	0.36	No early cohesiveness
5	0.66	Early cohesiveness
6	0.70	Early cohesiveness
7	0.75	Early cohesiveness

[0035]

[Effect of the Invention]

When the present invention is used, the early cohesiveness of the raw material wheat can be judged accurately and at good reproducibility in approximately 4 days. In addition, when the present invention is used, the early cohesiveness can be judged even for raw material wheat immediately after it has been harvested and even for small amounts of raw material wheat.